Day 44 - Monday - 8/4/14

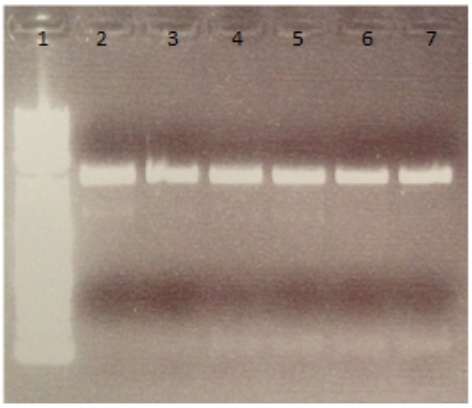
- In the morning, the team gave a presentation at a high school to educate students at a summer camp about synthetic biology and talk a little bit about our project.
- Alex and Chloe continued the western blot, making dilutions of GFP (1:1000), *E. coli* (1:1000), and CAEV (1:200) antibodies in milk, adding those to the appropriate membranes, and leaving them to rock for an hour. They were then rinsed 3 times in 1x PBS with Tween. Then, secondary antibodies diluted 1:10000 in milk were added to the membranes and left to rock for an hour. After that, they were rinsed three times in 1x PBS with Tween and prepared for film development.
- Mike Prepared two liquid cultures (26Ccol8, 32Ccol19) for use in this weeks GC/MS experimental trials.
- Corbyn and Shawna started the day by preparing and pouring a Western Blot gel with 4% stacking and 16% resolving gel. Then, we liquid cultured 6 colonies from the $35C_C$ (Promoter+RBS+BCLA) plate and labeled them $35C_{1\text{-}6}$. We also prepared 20uL cultures of 17K, $34C_{5,}25C_1$, and 9C and placed them in the shaker overnight. Lastly, we plated $30A_3$ (Promoter-RBS-BCLA-YFP) and $34C_5$ (Promoter-RBS-BCLA-YFP-DT) with the intention of giving the plates to BU to be used in flow cytometry experiments.

Day 45 - Tuesday - 8/5/14

- Alex and Chloe prepared a plate for an agglutination assay by adding the BSA and antibodies but not adding the bacteria and storing the plate in moist kimwipes and saran wrap. All units are in ul, and every dilution from 1:50 to the final concentration of 1:102400 was performed via serial dilution beginning at the 1:50 concentrations resulting in 50 ul in each well.

Strain	1:50	1:100
Untransformed E. Coli	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
BclA-CAEV	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
GFP	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
BclA-YFP	99 BSA 2 CAEV Antibody	50 BSA 50 From previous well
Untransformed E. Coli	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
BclA-CAEV	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
GFP	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well
BclA-YFP	99 BSA 2 GFP-8H11 Antibody	50 BSA 50 From previous well

- Kayla and Mike prepared all 18 liquid cultures outlined in the GC/MS Baseline Preparation protocol and placed them in the shaker to allow them to grow overnight.
- Corbyn and Shawna miniprepped and test digested (E-P) the liquid cultures of $35C_{1-6}$ (Promoter+RBS+BCLA) that had been shaking overnight. The resulting gel can be seen below with labeled lanes.



Lane	Contents
1	Ladder
2	35_C Sample 1
3	35_C Sample 2
4	35_C Sample 3
5	35_C Sample 4
6	35_C Sample 5
7	35_C Sample 6

Based on the faint lines at about 100bp in lanes 4,5,6, and 7, we sent $35C_{3,4,5,6}$ for sequencing. Due to failed plating from the previous day(a lawn), Corbyn and I replated $30A_3$ and 34C for BU. Lastly, we had to create more 20uL liquid cultures of 17K, $34C_{5,}25C_1$, and 9C because the cultures from the previous day had not grown due to the shaker having a much higher temperature than the 37degrees Celcius it was set to.

As a team, we also edited our presentation for NEGEM 3.2 as well as practiced our presentation.

Day 46 - Wednesday- 8/6/14

NEGEMMMMMMMMMMMMMM

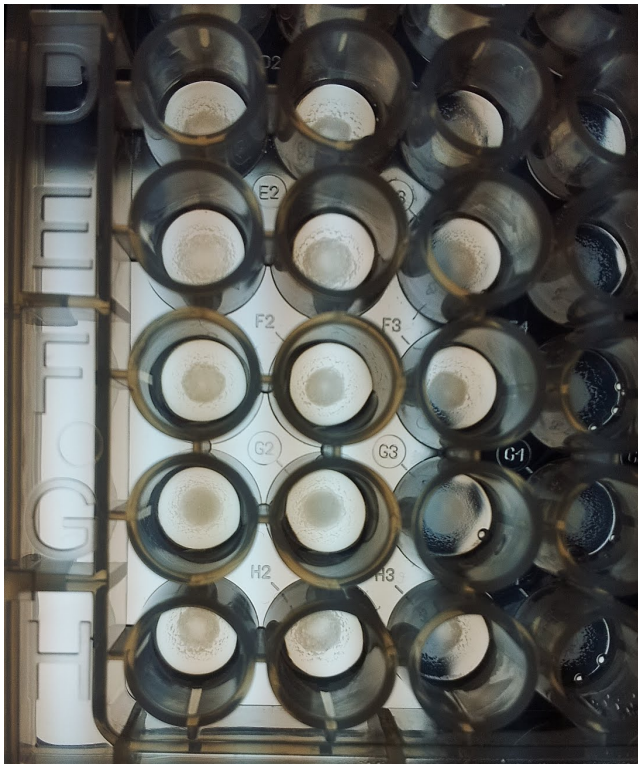
- In the morning before leaving for NEGEM, Corbyn and Shawna pelleted the 20uL liquid cultures from 17K, $34C_{5,}25C_1$, and 9C. We then stored the pellets at 20 degrees Celcius, with the intention of using the pellets in cell fractionation the next day. We also collected the $30A_3$ and 34C plates and brought them along with us to BU. Also, Corbyn viewed the sequencing results for $35C_{3,4,5,6}$ and found that the ligations were unsuccessful.

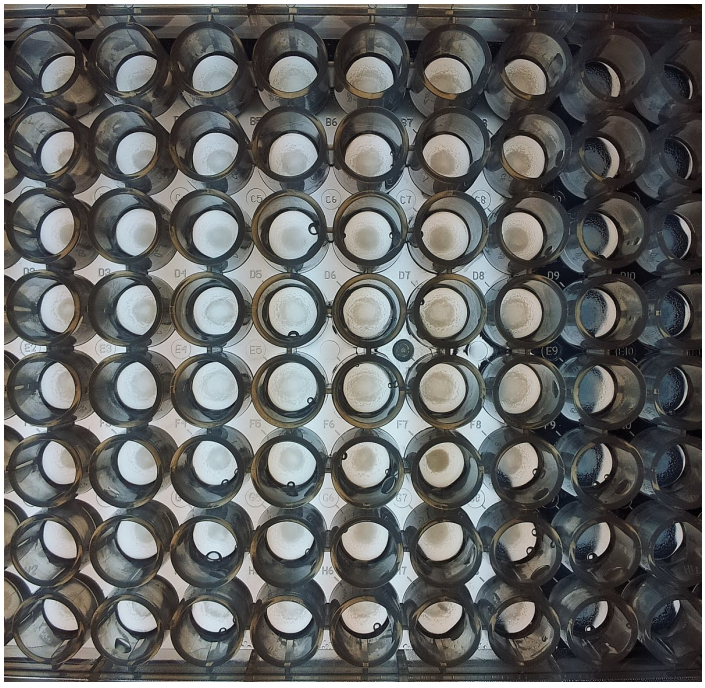
Day 47-Thursday-8/7/14

- Kayla and Mike prepared 19 vials for the first GC/MS trial. The samples were delivered to Andy Butler in the morning and then tested for most of the day. While the GC/MS experiment was running, 23 liquid cultures were prepared so another trial can be done tomorrow. The list of all the liquid cultures that were made can be seen in the GC/MS

Baseline Preparation protocol. Unfortunately, one of the liquid stocks was spilled, so the liquid cultures that were made were 1.5 mL instead of 5mL.

- The agglutination plate set up by Alex and Chloe appeared inconclusive.





- Kayla and Mike prepared 24 vials for the GC/MS experiment. Samples were delivered to Andy Butler at 10 am and the first sample injection occurred at approximately 10:15.

- Due to the failure to create the Promoter+RBS+BCLA biobrick(35C) , Corbyn and Shawna started the morning by setting up 50uL digests of the BCLA BB (E-X) and $28A_3$

(E-S) for 4 hours in the 37 degrees Celcius water bath. While allowing those to digest, we performed fractionation of the 17K, $34C_{5,}25C_{1}$, and 9C pellets, which we resuspended in 5ml of lysis buffer, using the sonicator. [finalize protocol and put into protocol folder].